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14. ABSTRACT
Many Navy wastewaters contain complex mixtures of metals and oils/greases, which are extremely difficult to treat. Biopraxis is developing a proprietary approach, based on particulate MOP-UP® reagents, already shown to be capable of taking heavy metals to sub-parts per billion (ppb) levels in complex freshwater wastes such as shipyard stormwater runoff. These MOP-UP® reagents have extremely high loading capacities; therefore, fewer consumables are needed, and far less secondary waste is produced. The simple production method will make it possible to manufacture reagents in bulk at very low cost. By coupling MOP-UP® reagents with off-the-shelf separation systems, economical, user-friendly, wastewater treatment systems can be produced. The objective of the Navy Wastewater MOP-UP®program was to evaluate the feasibility of using MOP-UP® to treat heavy metals and oils/greases in saline wastewaters. A variety of reagents screened against copper and zinc in artificial seawater were shown to take the metals to nondetectable levels, and to take up as much as 450 mg Cu/g reagent and 1,030 mg Zn/g. They were also very effective against representative components of oils and greases (alkanes, BTEX, and PAHs).

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FINAL REPORT

GRANT #: N00014-00-C-0359

PRINCIPAL INVESTIGATOR: Ann E. Grow

INSTITUTION: Biopraxis, Inc.

GRANT TITLE: Navy Wastewater MOP-UP®

AWARD PERIOD: 17 April 2000 - 16 April 2003

OBJECTIVE: Many Navy wastewaters contain complex mixtures of metals and oils/greases, which are extremely difficult to treat. Biopraxis is developing a proprietary approach in which microorganisms are used to produce reagents capable of taking heavy metals to sub-parts per billion (ppb) levels in complex freshwater wastes such as shipyard stormwater runoff. These MOP-UP® reagents can exhibit extremely high loading capacities; therefore, fewer consumables are needed, and far less secondary waste is produced. The simple production method will make it possible to manufacture reagents in bulk at very low cost. By coupling MOP-UP® reagents with off-the-shelf separation systems, economical, user-friendly, wastewater treatment systems can be produced. The objective of the Navy Wastewater MOP-UP®program was to evaluate the feasibility of using MOP-UP® to treat heavy metals and oils/greases in saline wastewaters.

APPROACH: Very little is known about MOP-UP® reagents - precisely what they are, the mechanisms that enable them to treat metals so effectively, or even the range of different reagents that can be produced. Therefore, the program consisted of several tasks. First, diverse reagents were tested for efficacy against copper and zinc in seawater. Properties that will dictate the separation systems that can be used (e.g., metal uptake kinetics, and reagent density and size) were measured. Simple studies to begin characterizing the reagents were performed. The degree to which reagent properties are sensitive to production parameters was evaluated. Finally, reagents were screened for their ability to treat representative components of oils and greases (alkanes, BTEX, and PAHs).

ACCOMPLISHMENTS: For MOP-UP® reagents to be effective and economical for use in treating seawater, the reagents must have a very high affinity (i.e., be capable of removing all detectable traces) and very high loading capacities for metals in a high-ion environment. A series of reagents that had proven to be effective in freshwater were screened in artificial sea water (ASW) spiked with 3ppm of copper or zinc. Generally speaking, a high salt content did not adversely affect reagent performance. In fact, many reagents were capable of taking both metals to nondetectable levels in ASW. Several appeared to interact with copper and zinc via different reaction mechanisms, exhibiting a much higher avidity for copper that was significantly less sensitive to pH. The reagents were also tested against excess metal ion solution (e.g., 250 or 500ppm), to evaluate the impact of a high-ion environment on loading capacities. Interestingly, ASW often *improved* the loading capacities. There was no clearly identifiable trend; some reagents exhibited higher loadings for copper and/or zinc in ASW than in MilliQ, some exhibited lower loadings, and some remained unaffected.

Two approaches are under consideration for separating MOP-UP® reagents from treated effluents; i.e., Memtek, a cost-effective microfiltration system, and Actiflo®, a high-flow-rate 'microsand' settling system. The costs for microfiltration systems are driven by the size of the particles to be separated, whereas reagent compatibility with Actiflo® will depend on particle density and reaction kinetics. Therefore, the sizes of the particles in select MOP-UP® reagents were measured before and after incubation in metal ion solutions; and copper removal kinetics were evaluated. The particles in a given reagent were tightly clustered in size; however, different reagents differed significantly, from reagents comprising particles 2-5µm in size, to those with very large (20µm in diameter and 50-70µm long) particles. Incubation in dilute metal ion solutions appeared to cause the particles to clump together; while incubation in concentrated solutions caused the particles to become larger (e.g., particles ~2x7µm in size grew as large as ~20x50µm in size), and sometimes became much more uniform in size and shape. There were no noticeable differences between a reagent incubated in copper and that same reagent incubated in zinc (aside from color.) Copper removal kinetics experiments indicated that (a) the initial reaction kinetics were very rapid; (b) the reaction slowed as the metal ion concentration became the controlling factor (i.e., became more and more dilute) and/or the pH went down significantly; and (c) as long as the metal ion concentration remained reasonably high, the reagents continued to take the metal up very rapidly over the 4-hour-long test.

Studies were initiated to determine how sensitive reagent properties may be to key production parameters. Five cultures were chosen for study; and two variables for each of two production parameters (carbon source and the concentration of a nutrient supplement) were altered for each, resulting in a matrix of four 'recipes' from each culture. The impact of these parameters was studied by evaluating the physical (size, shape, color) and chemical properties of the reagent particles; and the ability of the reagents to treat copper, zinc, and oils/greases.

The production parameters had far more impact than expected. It was originally assumed that, since the production system is homogeneous, the particles produced by a given recipe would differ at most only in size. However, a given recipe was found to contain as many as four distinctly different particles; and different recipes used with the same culture produced dramatically different particles. They ranged from irregular 1 µm black particles, to 15-20µm peach balls with undulate surfaces (Fig. 1), to pale green balls clustered into 30µm globes, to translucent grey spheres as large as 100 µm in diameter, uniformly covered with tiny bumps.

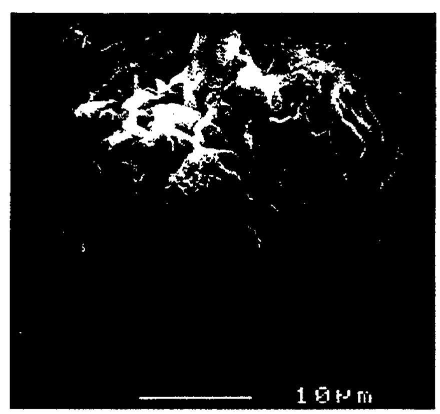

Figure 1. Example of an 'undulate peach ball' particle

The discovery that a given recipe could produce more than one type of particle severely limited the analytical methods that could be used to characterize the chemical properties of the reagents, since the methods had to have a spatial resolution of ~1μm to collect data from individual particles. Raman microscopy, electron microprobe (EDX), and scanning electron microscopy (SEM) were used. Since the EDX data were collected at the same time as the SEM, information on the elemental composition could readily be matched with a given particle type. And, for the most part, it was easy to match Raman spectra with SEM/EDX analyses, since most particles looked the same whether under the light microscope, the microRaman, or the SEM. In a few instances, new types of particles were discovered when the EDX and Raman results did not appear to match, and the reagent was re-examined. Significantly, the Raman and EDX spectra could not be readily correlated to standard spectra of known materials. This was expected, since MOP-UP® reagents exhibit highly unusual properties. Certain peak assignments could be tentatively made, however.

Generally speaking, there were no significant differences between Raman spectra from reagents rinsed in water vs those rinsed in ASW, aside from a small peak in the salt-rinsed spectra that was tentatively assigned to one of the salts in the rinse. Raman spectra from reagents incubated in anaerobic samples did not contain peaks that were seen when incubated in oxygenated samples, indicating that reagent properties may be sensitive to oxygen. Raman spectra from reagents heavily loaded with copper and/or zinc were compared to those from lightly-loaded reagents. In general, the change in the spectrum from a given reagent was more intense if that reagent took up a higher metal loading; otherwise, the spectra were similar. Tentative assignments were made for some of the oxidation and metal-related peaks. When reagents were incubated in solutions of zinc, nickel, copper, or cadmium and then centrifuged and examined under a microscope, the different metals caused the pellets to turn different colors, indicating that reactions had taken place. However, the Raman spectra did not always correlate with the color changes.

All twenty recipes were tested for efficacy against copper and zinc. Nine took copper to nondetectable levels; six took zinc to nondetectable levels; and four of these were effective against both. Under the static (no agitation) 4-hour incubation test used, copper loadings were as high as 460mg Cu/g reagent, and zinc loadings as high as 1,030mg Zn/g reagent - i.e., far higher than seen with conventional adsorbents. One recipe performed exceptionally well, taking both metals to nondetectable levels and taking up high loadings of both, i.e., 450 mg Cu/g and 1,030 mg Zn/g. There were no clear patterns as to the production parameters that resulted in superior heavy metal treatment efficacy.

Several recipes produced mixtures of gray and peach particles. It appeared that the peach particles might have been primarily responsible for taking up copper, since reagents with a higher peach content were more effective against copper, and peach particles turned black after incubation in copper, whereas the gray particles remained unchanged. A sucrose gradient centrifugation method was used to fractionate a recipe comprising ~50% grays and ~50% peaches. As expected, the copper loading achieved by the exclusively peach fraction high was far higher than the other fractions. Surprisingly, however, the exclusively gray fraction was more effective at removing zinc. Raman spectra indicated that the particles might be reacting with the sucrose when separated under air. When separated under a nitrogen environment, the loadings achieved by the peach particles did not appear to be affected. The gray particles, on the other hand, achieved higher loadings of both copper and zinc when exposed to air during sucrose gradient centrifugation.

All twenty recipes were also tested for their ability to treat oils and greases, using 500ppm benzene, toluene, and n-nonane, and saturated solutions of naphthalene. The best removal rates were 866 mg/g n-nonane; 446 mg/g benzene; 336 mg/g toluene; and 23% naphthalene - again, much higher than seen with conventional adsorbents. Generally, the contaminants most responsive to treatment were n-nonane >> benzene >> toluene. However, one recipe treated benzene much more readily than n-nonane, while two others treated twice as much toluene per gram as benzene. To determine whether oxygen might have any impact, one culture was used to prepare the matrix of four recipes, which were tested for efficacy against benzene. All four recipes tested under oxygenated conditions were effective at treating benzene, and followed the same trends seen in the previous experiments, with one recipe treating 304 mg benzene/g. None of the four tested under anaerobic conditions was able to remove any benzene at all.

CONCLUSIONS: MOP-UP® reagents exhibited excellent efficacy against at least one, and typically several, different metal and/or organic contaminants. The level of performance that was seen against both metals and organics was far superior to that seen with conventional adsorbents. The production parameters can be tailored to improve efficacy even more. Studies have shown that different reagents with diverse properties can be mixed together, and are even better at treating complex contaminant mixtures than any reagent alone. MOP-UP® therefore promises to be a very effective and economical solution for treating environments contaminated with multiple pollutants.

SIGNIFICANCE: Compact, economical systems capable of treating Navy wastewaters for direct discharge will be of value for treating underwater hull cleaning wastewater; surface vessel and submarine bilgewaters; clean, dirty, and compensated fuel ballasts; seawater cooling overboard discharge; and deck runoff. Treatment systems could also be used to clean up accidental spills; and to treat metalworking wastewaters, e.g., from electroplating activities.

PATENT INFORMATION: Biopraxis filed patent applications prior to submitting the proposal to ONR, which are in process.

PUBLICATIONS AND ABSTRACTS: Papers will be published once the patents have started to issue.